Response to Chemical Cues From A Predator In A Cavity-Nesting Bird Species, The House Wren (*Troglodytes aedon)*

Research Thesis

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By

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Abstract

Birds have long been considered to have poor senses of smell, but recent studies have shown differential behavioral reactions to various scents in songbirds. Predator detection via olfaction may be particularly important in cavity nesters because predators could trap them or ambush them from within the cavity. We examined the response of House Wrens (*Troglodytes aedon)*, a common, cavity-nesting songbird, to a predator scent. A previous study found that wrens did not respond during the nestling feeding stage, and we hypothesized that the wrens may show anti-predatory behaviors in relation to their investment in the offspring. Specifically, we predicted that wrens may be more hesitant during the incubation stage than the nestling feeding stage. To test this, we placed filter papers treated with urine from the American mink (*Neovison vison)*; a pureed solution of garlic mustard (*Alliaria petiolata)*, an odiferous control; or water in the nest box during both the incubation and feeding stages. We video-recorded the response of the wrens and quantified changes in time to enter the box or time spent in the nest box. Our data showed no significant differences in the reaction of wrens during the incubation stage; during the feeding stage however, there was a decrease in the longest visit to the box in order from the garlic scent, the mink scent, to the water control. Our results indicate that although wrens may not use olfaction to avoid predators, they do modify their behavior in the presence of certain smells.

Introduction

A classic example of coevolution is the evolution of predators to detect and capture prey and the evolution of prey to detect and elude predators. Depending on the prey species, predator detection may occur via sight, sound, vibration or olfaction. Upon detecting a predator, the prey exhibits anti-predatory behaviors (Kats & Dill 1998), such as a decrease in movement, an increase in vigilance, or relocation to a safer environment (Apfelbach *et al.* 2005).

The role of olfaction for predator detection is relatively little-studied compared to predator detection via sight or sound. Olfaction may play a particularly significant role during poor visual and auditory occasions (Zidar & Løvlie 2012), giving organisms an alternative means of environmental awareness. Exposure to predator chemical cues has shown to stimulate anti-predatory behaviors in fishes (Ylönen *et al.* 2007), invertebrates (Thomas *et al.* 2008), reptiles (Amo *et al.* 2004), and mammals (Apfelbach *et al.* 2005); however, until recently, few studies have focused on olfaction in birds (Kats & Dill 1998).

This lack in avian studies on predator detection via chemical cues may be a result of the common assumption that most birds have a poor sense of smell (Kats & Dill 1998). However, recent studies have shown the use of olfaction in birds for foraging, navigation, selection of nest materials, and species identification (Roth *et al.* 2008; Zidar & Løvlie 2012). Therefore, it would seem that an evaluation of predation risk via detection of chemical cues would be beneficial for birds, especially when entering a cavity or during events of poor visibility (Amo *et al.* 2008). This has led to a resurgence of research on the use of olfaction in birds to detect predators.

The results of avian studies examining predator detection via olfaction have been inconclusive. House Finches (*Carpodacus mexicanus)*, Pied Flycatchers (*Ficedula hypoleuca*), and Blue Tits (*Cyanistes caeruleus*) showed anti-predatory behavior when exposed to predator mammal scents (Roth *et al.* 2008; Mönkkönen *et al.* 2009; Amo *et al*. 2008). However, Eastern Bluebirds (*Sialia sialis)* and House Wrens (*Troglodytes aedon)* did not show a response to predator scents (Godard *et al.* 2007; Johnson *et al.* 2011). It is therefore important to further investigate the extent to which birds use smell to detect predators.

In this study, we conducted an experiment similar to those of Johnson *et al.* (2011) and Amo *et al.* (2008), and further investigated the response of House Wrens to the odor of a common predator previously observed at our study site, the American mink (*Neovison vison)*. These previous studies only examined responses during the nestling feeding stage; we also examined the responses during the incubation stage, because we hypothesized that the wrens may show anti-predatory behaviors in relation to their investment in the offspring (Redmond *et al.* 2009; Andersson *et al.* 1980). Specifically, we predicted that wrens may be more hesitant during the incubation stage than the nestling feeding stage. We placed scents of mink urine, garlic mustard (scent control), and water (unscented control) in nest boxes during the incubation and nestling feeding stages, and recorded the time taken to approach the box after the researcher left, the time spent from approach until entry, and the total time spent in the box. We modeled our experiments after Johnson *et al.* (2011) who examined the wren's behavior after the addition of a scent during the nestling feeding stage. We expanded this study by

recording the wren's behavior during the incubation stage, as well as controlling for the wren's behaviors before the addition of the scents.

Material and Methods

Study Species

House Wrens are small, cavity nesting, migratory passerines that are commonly found throughout North America. They display a brownish gray plumage, and measure between 11-13 cm and 10-12 g (Johnson 1998). Nesting typically occurs in dead tree cavities, old woodpecker holes, or man-made nest boxes (Johnson 1998). House Wrens are typically found on the ground or in the subcanopy preying on insects (Mirsky 1976). Females generally lay two clutches during the breeding season (Kendeigh 1963). The first clutch is laid in early May (Robinson & Rotenberry 1991), with an average size of 7 eggs (Newhouse *et al.* 2008). If there is a second clutch, it generally occurs in early July (Robinson & Rotenberry 1991), with an expected average size of 6 eggs (Newhouse *et al.* 2008). Both sexes give parental care for nestlings (Johnson 1998). However, in an attempt to breed elsewhere, males have been seen to abandon nestlings (Czapka & Johnson 2000). Common predators of wrens are cats (*Felis* spp.), weasels (*Mustela* spp.), squirrels (*Sciurus* & *Tamiasciurus* spp.), raccoons (*Procyon lotor*), foxes (*Vulpes* spp.), rat snakes (*Elaphe* spp.) and garter snakes (*Thamnophis* spp.) (Kendeigh 1942; Johnson 1998).

Study site and field techniques

A total of 120 nest boxes were placed between April and August 2013 in three different locations: a woodland habitat, which is located on the Ohio State Lima campus

(40.743338, -84.017129); a park, which is also located on the Ohio State Lima campus (40.736298, -84.029939); and a golf course (40.75551, -84.026012). The golf course was located less then 5 km from the Ohio State Lima campus. The nest boxes in the forest were adjacent to natural grassland vegetation, those in the park were located along tree lines surrounding the area, and those at the golf course were placed along a shrubby fencerow that surrounded the course. The nest boxes measured 14.0 x 10.1 x 20.3 cm, with an opening 2.5 cm below the top of the box measuring 2.9 cm in diameter. The boxes were spaced ≥30 m apart to account for male territoriality (Muller *et al.* 1997).

All nest boxes were checked every 3-4 days from the end of April until early August. When there was >4cm of nesting material in the box, the box was checked daily to determine the exact date that the first egg was laid. Eleven days after two consecutive days with no increase in the number of eggs, the nest was checked daily to observe hatching. This ensured that we would determine the exact hatching date, defined as the date when >50% of the eggs hatch. The frequent checks also helped to provide information on clutch size, hatching success for both the first and second clutches, and the time interval between clutches.

Odor-detection experiment

Following Johnson *et al.* (2011) and Amo *et al.* (2008), pieces of filter paper (9 cm in diameter) were used to contain odors. Two pieces of paper were placed in nest boxes on the left and right side, and the reaction of the adult birds were recorded with a video camera. To keep the filter papers themselves from having a major effect on the bird's behavior, two untreated pieces of paper were placed in the nest box during nest

construction. When an odor was tested, the untreated papers were replaced with the experimental papers. After testing, the untreated papers were returned.

Experimental testing occurred on days 5-6 of incubation and day 4 after hatching with one of three different odors. The urine of the American mink was used as a predator odor (obtained from Molnar Outdoor, Elyria, OH). Even though mink are not common nest predators, their urine is probably similar in smell to other closely related mustelids, such as the long-tailed weasel (Johnson *et al.* 2011). Molecular-based phylogeny also suggests that the long-tailed weasel is more closely related to the American mink than any other weasels (Koepfli *et al.* 2008). Garlic mustard, which can be found on site, was used as a control odor. The leaves of the garlic mustard were ground up into a paste. To ensure that odor intensities were equal, we diluted the garlic paste with water to match intensity of the mink urine. As an odorless control, water was used to maintain a consistency with paper wetness and humidity levels. Papers were infused with 5 mL of a scent and put in a small (16.5 cm x 14.9 cm) sealed plastic bag overnight. Each box was given one treatment with the initial order of the treatments randomized and repeated every 3 boxes. Different treatments were given during the incubation and feeding stages. Some boxes were depredated between the incubation and feeding stages, but we tried to maintain an equal number of treatments within each stage.

A Sony HDR-CX160 video camera was used to record the response of adult birds. During the incubation stage, we recorded the bird's response for 30 min before the treatment and for 30 min after the placement of the scent infused papers or until the bird left the box (which ever came first). During the feeding stage, we recorded the birds for 20 min before the treatment and for 20 min after the placement of the scent infused

papers. The camera had a 40x optical zoom, so it was placed 30-50 m away and should not have caused any disturbance to the nest.

Data Analysis

From the video recording timescale, we recorded the time the researcher left the box, the time a wren landed on the box, and the time a wren entered and exited the box (to the nearest second). During the feeding stage, we also recorded the number of times the wrens visited the box within a 10 min period. From these times, we determined the time taken to approach the box after the researcher left, the time spent from approach until entry, and the time spent for the first visit. Also during the feeding stage, we determined the total time spent in the box in 15 min and the longest visit to the box (defined as the longest time spent for one visit). The longest visits to the box during the incubation and nestling feeding stages were of interest because this represents the time the female is brooding the eggs or nestlings, respectively. During the incubation stage, we excluded two boxes from the analysis, because the female removed the scent (garlic) filter paper. It was expected that if the scent was detected, wrens would delay their entry into the box and spend less time in the box.

To compare the wren's behaviors before and during the treatment, a matched pairs analysis was used. When necessary, we used a student's t-test to determine when responses were different between scents. All analyses were conducted in JMP (Version 9.0.0, SAS Institute Inc.).

Results

There were 109 overall nest attempts out of the 120 nest boxes available. Of these attempts, 53% were successful, and 47% were unsuccessful. Experiments were conducted on 75 boxes during incubation and 73 boxes during nestling feeding. Some boxes were depredated or abandoned before incubation, or between incubation and nestling feeding, and so we could not conduct an experiment on every nest attempt.

Incubation Stage

Of the 75 boxes in which experiments were conducted during the incubation stage, 26 were given the mink scent, 25 were given the garlic scent, and 24 were given the water control. Wrens did not show any difference in their behavioral response when exposed to various scents (Table 1). No difference was seen in the time to approach the nest box ($F=0.35$, $N=60$, $P=0.71$), the time to enter the nest box ($F=0.72$, $N=75$, $P=0.49$), and the visit length in the nest box ($F=0.60$, $N=75$, $P=0.55$).

Nestling Feeding Stage

Of the 73 boxes in which experiments were conducted during the nestling feeding stage, 22 were given the mink scent, 23 were given the garlic scent, and 28 were given the water control. Wrens did show some differences in their behavioral response when exposed to various scents (Table 1). Wrens had the shortest visit to the box when garlic scent was used and had the longest visit to the box when water was used (Figure 1; $F=4.42$, $N=72$, $P=0.02$). However, other behavioral responses did not vary according to scent used during the nestling feeding stage including the time to enter the nest box ($F=0.16$, $N=46$, $P=0.85$), the total time spent in the nest box ($F=1.66$, $N=73$, $P=0.20$), and the number of times the wrens visited the nest box in 10 min ($F=0.12$, $N=73$, $P=0.89$).

Discussion

Our results suggest that House Wrens may be capable of smelling, because they shortened the amount of time they spent in the box when garlic scent was used during the nestling feeding stage. It seems surprising that wrens showed the greatest response in the presence of the garlic mustard scent, because it is a plant common to the area and frequently found around the nest boxes. However, garlic mustard contains glucosinolates, which are responsible for the pungent odor, that are released when the plant is crushed (Daxenbichler *et al.* 1991; Vaughn & Berhow 1999). It is therefore possible that wrens associate the smell of garlic mustard with the presence of a predator, as the predator would crush the plant as they trample or brush over it. However, further research would be necessary to verify that predators crush the garlic mustard to the extent that would be necessary for wrens to detect.

We had originally thought that wrens would be more hesitant to enter the box and spend less time in the box during the incubation stage than during the nestling feeding stage, due to less investment in the clutch. The parental investment theory predicts that parents will take more risk with increased brood size and age (Redmond *et al.* 2009; Andersson *et al.* 1980). We, however, did not see any difference in wren's behaviors to the scents during the incubation stage, like we had predicted.

In a similar study, Johnson *et al.* (2011) did not find any anti-predatory behaviors or any difference in behaviors when House Wrens were exposed to mink urine as a predator scent, a garlic scent, as well as cologne as an odiferous control scent, and water as a control. We may have detected a response to the scents because we controlled for the

wren's behaviors before the addition of the scent, whereas the Johnson study only examined the wren's behaviors after the addition of the scent.

Other studies have shown differential behaviors in other cavity-nesting songbirds. Amo *et al.* (2008) found that Blue Tits delayed their entry into the box, and spent less time in the box when presented with a predator scent (mustelid). We however did not see as strong of a reaction in House Wrens. In our study, wrens reacted highly to the garlic scent, and we did not see any difference in the time taken to enter the box. Like House Wrens, Eastern Bluebirds did not avoid nesting in boxes that contained chemical cues from common nest predators, the black rat snake (*Elaphe obsolete*) and the deer mouse (*Peromyscus maniculatus*) (Godard *et al.* 2007). Three factors may influence whether birds respond to scents. First, some birds may experience a stronger threat from predators, which would place a stronger pressure on them to evolve a better sense of smell. Second, it is possible that some birds have a better sense of smell than other birds; therefore, they can more easily detect and distinguish among predator scents. Third, olfaction may not offer an evolutionary advantage during predator detection, but olfaction might be important in other contexts such as foraging or species identification (Roth *et al.* 2008; Zidar & Løvlie 2012).

Future studies should examine individual responses to different scents. We were not able to identify the sex of the individual on the video recordings, so future work may investigate whether females or males respond differently to the scents. It is most likely that females would show a stronger response than males, because female wrens spend more time in the box for incubation and feeding and they are more assured of paternity;

therefore, if olfaction is used to detect predators, it would seem to be more advantageous for females.

In conclusion, our results suggest that House Wrens may not necessarily use smell to detect predators, but they do have the ability to detect smell, and can alter their behavior when exposed to a foreign scent. This study contributes to the growing body of research showing that birds do indeed have a sense of smell. Further research should investigate the extent to which different species use smell to enhance their fitness.

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Figure 1: The difference between the longest time spent in the box before the placement of the scent and after. Different letters represent significant differences ($P<0.05$) using a student's t-test.

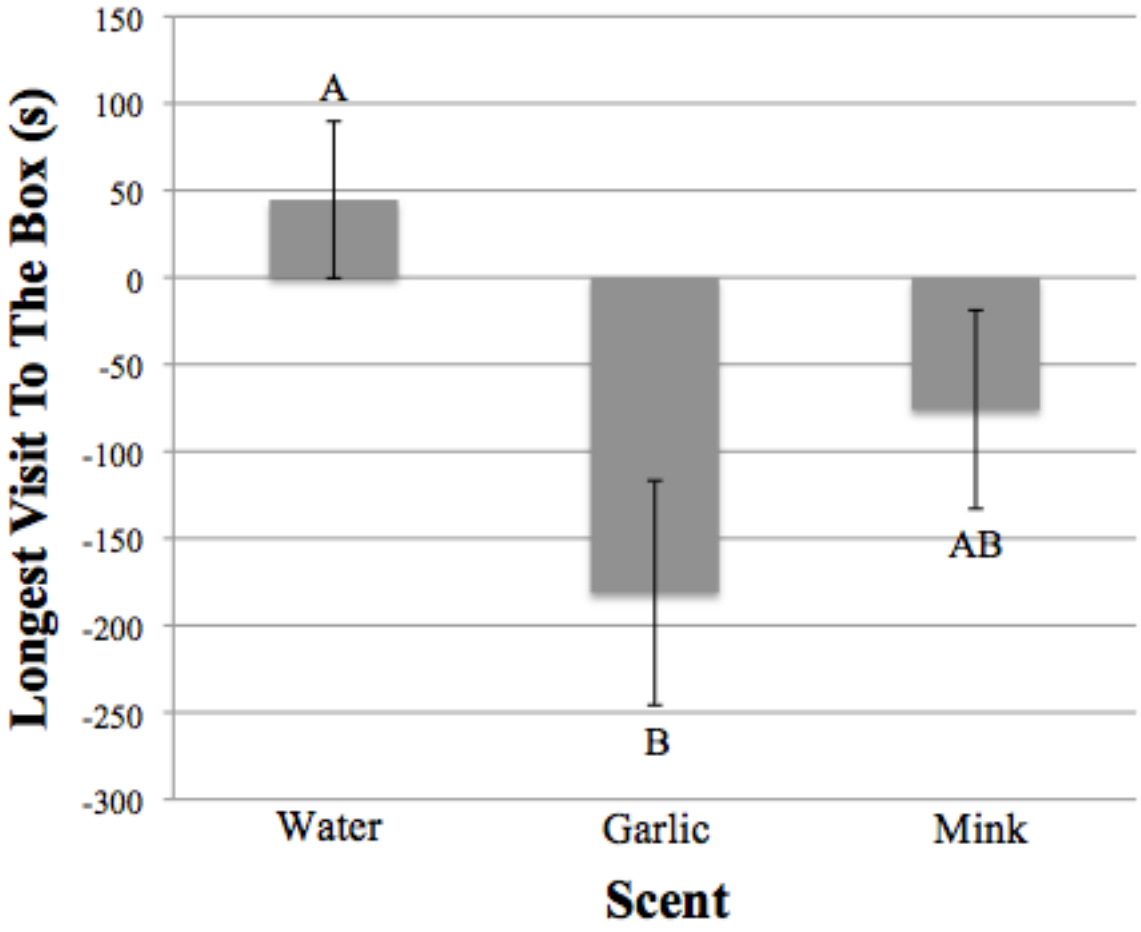

Table 1: The mean difference ± the standard error in response of House Wrens before and during the presence of experimental or control scent cues. A negative value means that the variable was shorter after the addition of the scent than before.

Explanatory Variables	Incubation Stage	Nestling Feeding Stage
Time to approach (s)	-33.4 ± 47.4	
Time to enter (s)	2.2 ± 1.4	3.0 ± 1.2
Visit length (s)	-105.8 ± 52.2	-61.2 ± 33.1
Total time spent in box (s)		-6.1 ± 30.1
Number of visits in 10 min		-0.3 ± 0.2